

A SURVEY OF COMPUTATIONAL METHODS FOR PROTEIN COMPLEX PREDICTION FROM PROTEIN INTERACTION NETWORKS

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Complexes of physically interacting proteins are one of the fundamental functional units responsible for driving key biological mechanisms within the cell. Their identification is therefore necessary not only to understand complex formation but also the higher level organization of the cell. With the advent of “high-throughput” techniques in molecular biology, significant amount of physical interaction data has been catalogued from organisms such as yeast, which has in turn fueled computational approaches to systematically mine complexes from the network of physical interactions among proteins (PPI network). In this survey, we review, classify and evaluate some of the key computational methods developed till date for the identification of protein complexes from PPI networks. We present two insightful taxonomies that reflect how these methods have evolved over the years towards improving automated complex prediction. We also discuss some open challenges facing accurate reconstruction of complexes, the crucial ones being presence of high proportion of errors and noise in current high-throughput datasets and some key aspects overlooked by current complex detection methods. We hope this review will not only help to condense the history of computational complex detection for easy reference, but also provide valuable insights to drive further research in this area.

Keywords: Protein Complex Prediction; Protein Interaction Network; Sparse Complexes

1. Introduction

Most biological processes within the cell are carried out by proteins that physically *interact* to form stoichiometrically stable *complexes*. Even in the relatively simple model organism *Saccharomyces cerevisiae* (budding yeast), these complexes are comprised of many subunits that work in a coherent fashion. These complexes interact with individual proteins or other complexes to form functional modules and pathways that drive the cellular machinery. Therefore, a faithful reconstruction of the entire set of complexes (the ‘complexosome’) from the physical interactions among proteins (the ‘interactome’) is essential to not only understand complex formations, but also the higher level cellular organization.

Protein complexes constitute *modular* functional units within the network of physical interactions, the PPI network¹. From a biological perspective, this mod-

ularity is a result division of labor and of evolution to provide robustness against mutation and chemical attacks². From a topological perspective, this modularity is a result of proteins within complexes being densely connected to each other than to the rest of the network³.

Since the advent of “high-throughput” screening in molecular biology in the late 1990s and early 2000s, several techniques have been introduced to infer physical interactions among proteins within organisms in a large-scale (“genome-wide”) manner. These have helped to catalogue significant amount of protein interactions in organisms such as yeast thereby fueling computational techniques to systematically mine and analyze such large-scale interaction data. In yeast, the Yeast two-hybrid (Y2H)^{4,5}, Protein Complementation Assay (PCA)⁶ and Tandem Affinity Purification followed by Mass Spectrometry (TAP-MS)^{7,8,9,10} are some of the widely adopted experimental systems that have helped to identify a considerable fraction of physical interactions among proteins. However, even at the current ‘state-of-the-art’, these high-throughput techniques have been shown to produce considerable proportion of spurious (false positive) interactions^{11,12,13,14}. Therefore, once the interactions are identified their qualities need to be first assessed to generate a reliable set of interactions that is deemed suitable for further mining and analysis. This process includes assigning each interaction a confidence score that typically accounts for the biological variability and technical limitations of the experimental conditions, and therefore reflects the reliability of the inferred interaction^{15,16,17,18,19,20,21,22,23,24}. The interactions with confidence scores below a certain threshold are discarded to build a reliable “cleaned-up” PPI network. This PPI network is then mined to identify groups of proteins potentially forming complexes. The whole process can be summarized in the following steps:

- (1) Integrating high-throughput datasets from multiple experiments and assessing the reliabilities of interactions;
- (2) Constructing a reliable PPI network;
- (3) Identifying modular subnetworks from the PPI network to generate a candidate list of complexes;
- (4) Evaluating the identified complexes against *bona fide* complexes, and validating and assigning roles to novel complexes.

The identification of complexes from high-throughput interaction datasets has attracted considerable attention from both biologists as well as computational research communities, and over the years, several computational techniques have been developed to systematically identify complexes. Quite naturally, a number of surveys have come out from time-to-time evaluating and comparing these techniques for their performance on available PPI datasets. One of the earliest comprehensive evaluations was by Brohee and van Helden (2006)²⁵. This was followed by Vlasblom et al. (2009)²⁶ and Li et al. (2010)²⁷. While Brohee and Vlasblom et al. evaluated and compared some early methods on PPI datasets available at that time (till 2006), Li et al. covered some of the more recent methods developed until 2009.

The purpose of our work is to provide an up-to-date survey, classification (taxonomy) and evaluation of some the representative works done till date (2011/2012). We build upon the existing surveys so as to not repeat entirely the evaluations and conclusions already drawn, yet we provide our own classifications and evaluations of more recent techniques across up-to-date PPI datasets. We also compare across unscored (raw) and scored PPI datasets, which is missing in these existing surveys. We also highlight and comment on some of the newer challenges and open problems in complex prediction, which can guide future directions for research in this area.

2. Review of existing methods for complex detection

We begin by mentioning some definitions and terminologies widely adopted across the reviewed works. A PPI network is modeled as an undirected graph $G = (V, E)$, where V is the set of proteins and $E = \{(u, v) : u, v \in V\}$ is the set of interactions among protein pairs. For any protein $v \in V$, $N(v)$ is the set of direct neighbors of v , while $\deg(v) = |N(v)|$ is the degree of v . The interaction density of G is defined as $density(G) = \frac{2 \cdot |E|}{|V| \cdot (|V| - 1)}$. This is a real number between 0 and 1, and typically quantifies the “richness of interactions” within G : 0 for a network without any interactions and 1 for a fully connected network. The clustering coefficient $CC(v)$ measures the “cliquishness” of the neighborhood of v : $CC(v) = \frac{2 \cdot |E(v)|}{|N(v)| \cdot (|N(v)| - 1)}$, where $E(v)$ is the set of edges in the neighborhood of v . If the interactions of the network are reliability scored (weighted), these definitions can be extended to their corresponding weighted versions: $\deg_w(v) = \sum_{u \in N(v)} w(u, v)$, $density_w(G) = \frac{\sum_{e \in E} w(e)}{|V| \cdot (|V| - 1)}$, and $CC_w(v) = \frac{\sum_{e \in E(v)} w(e)}{|N(v)| \cdot (|N(v)| - 1)}$, where $w : E \times E \rightarrow \mathcal{R}$ is a scoring function on the interactions in E . There are several interesting variants proposed for weighted clustering coefficient CC_w ; for a survey see²⁸.

2.1. Taxonomy of existing methods

Although at a very generic level most existing methods make the key assumption that complexes are embedded among densely-interacting groups of proteins within PPI networks, these methods vary considerably either in the algorithmic methodologies or the kind of biological insights employed to detect complexes. Accordingly, we classified some popular complex detection methods into two broad categories (a soft classification): (i) methods based solely on graph clustering; (ii) methods based on graph clustering and some additional biological insights. These biological insights may be in the form of functional, structural, organizational or evolutionary information known about complexes or their constituent proteins from experimental or other biological studies.

We present this classification in two snapshots. The first snapshot, shown in Figure 1, gives a *chronology-based* “bin-and-stack” classification, while the second snapshot, shown in Figure 2 gives a *methodology-based* “tree” classification of the methods.

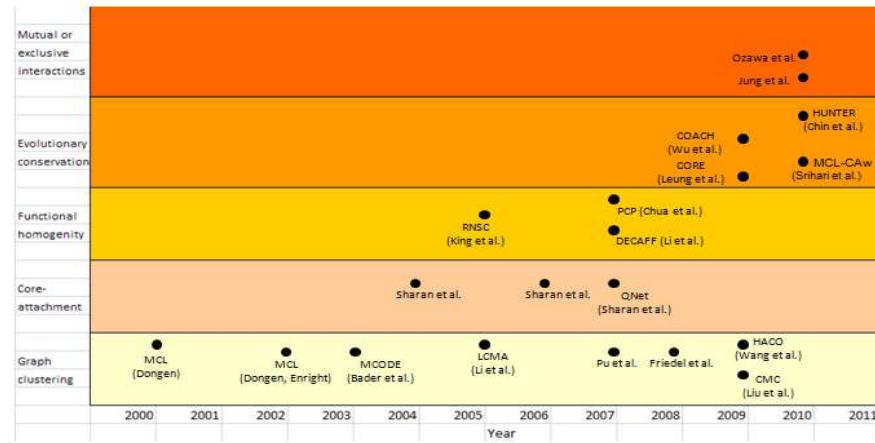


Fig. 1. The “Bin-and-Stack” classification: Chronological binning of complex detection methods based on biological information used. It is interesting to note that over the years, as researchers have tried to improve the basic graph clustering ideas, they have also incorporated biological information into their methods.

In the chronology-based classification, we *binned* methods based on the years in which they were developed, and stacked them based on the kind of biological insights used (see Figure 1). The biological insights are grouped as: core-attachment structure, evolutionary information, functional coherence, and mutually exclusive and co-operative interactions. It is interesting to note from this classification that, over the years, as researchers tried to improve the basic graph clustering ideas, they also incorporated a variety of biological information into their methods.

In the methodology-based classification, we distributed the methods to different branches of a *classification tree* based on the kind of computational strategy used (see Figure 2). At the first level from the root, we grouped these methods into those based solely on graph clustering, and those employing additional biological insights. At subsequent levels, we further divided these methods based on the kind of algorithmic strategies used, into: (i) methods employing merging or growing of clusters; (ii) methods employing repeated partitioning of networks; and (iii) methods employing network alignment. The methods employing merging or growing clusters go “bottom-up”, that is, typically start with small “seeds” (for example, triangles or cliques), and repeatedly add or remove proteins or merge clusters based on some similarity measures to arrive at the final set of complexes. On the other hand, the

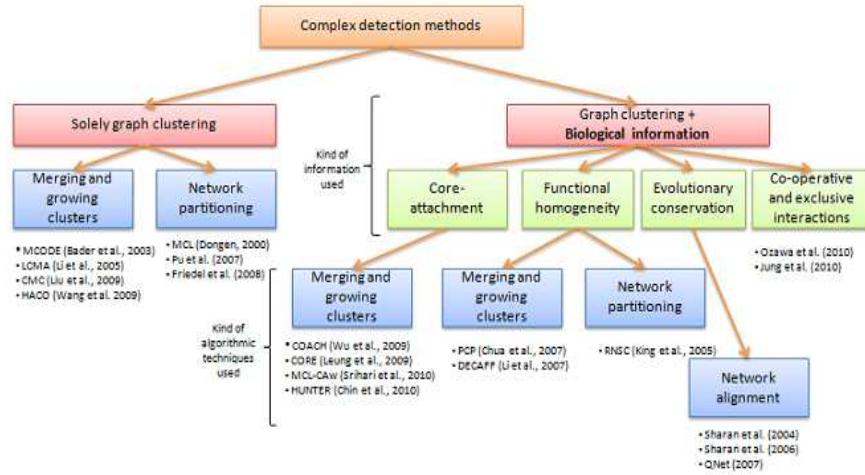


Fig. 2. The Tree classification: Classification of existing methods for complex detection based on the algorithmic methodologies used. Primarily three methodologies are adopted: merging and growing clusters, network partitioning and network alignment.

methods based on network partitioning go “top-down”, that is, repeatedly partition or break the network into multiple subnetworks based on certain divisive criteria. The methods based on network alignment use multiple networks (typically from different species) to arrive at isomorphic regions that likely correspond to complexes, the intuition being that proteins belonging to real complexes should generally be conserved through the evolution process to act as an integrated functional unit³.

2.2. *Methods based solely on graph clustering*

Most methods that cluster the PPI network into multiple dense subnetworks make use of solely the topology of the network.

Molecular COnplex DEtection (MCODE)

MCODE, proposed by Bader and Hogue (2003)²⁹, is one of the first computational methods (and therefore, seminal) developed for complex detection from PPI networks. The MCODE algorithm operates in mainly in two stages, vertex weighting and complex prediction, and an optional third stage for post-processing.

In the first stage, each vertex v in the network $G = (V, E)$ is weighted based on its neighborhood density. Instead of directly using clustering coefficient, MCODE uses core-clustering coefficient which measures the density of the highest k -core in the neighborhood of v . This amplifies the weighting of densely connected regions in G . In the second stage, the vertex v with the highest weight is used to seed a complex. MCODE then recursively moves outwards from the seed vertex, including vertices

into the complex whose weight is a given percentage (vertex weight parameter - VWP) away from the seed vertex. A vertex once added to a complex is not checked subsequently. The process stops when there are no more vertices to be added to the complex, and is repeated using the next unseeded vertex. At the end of this process multiple non-overlapping complexes are generated. The optional third stage performs a post-processing on the complexes generated from the second stage. Complexes without 2-cores are filtered out, and new vertices in the neighborhood with weights higher than a given ‘fluff’ parameter are added to existing complexes. The resultant complexes are scored and ranked based on their densities. The time complexity of the algorithm is $O(|V|.|E|.h^3)$, where h is the vertex size of the average vertex neighbourhood in the network G .

Markov Clustering (MCL)

The Markov Clustering (MCL) algorithm, proposed by Stijn van Dongen (2000)³⁰, is a general graph clustering algorithm that simulates random walks (called *flow*) to extract out relatively dense regions within networks. In biological applications, it was first applied to cluster protein families and ortholog groups³¹ before it proved to be effective in detecting complexes from protein interaction networks^{19,32,33}.

MCL manipulates the adjacency matrix of networks with two operators called *expansion* and *inflation* to control the random walks (flow). Expansion models the spreading out of the flow, while inflation models the contraction of the flow, making it thicker in dense regions and thinner in sparse regions. These parameters boost the probabilities of intra-cluster walks and demote those of inter-cluster walks. Mathematically, expansion coincides with normal matrix multiplication, while inflation is a Hadamard power followed by a diagonal scaling. Therefore, MCL is highly efficient and scalable. The iterative expansion and inflation separates the network into multiple non-overlapping regions.

Clustering based on merging Maximal Cliques (CMC)

CMC was proposed by Liu et al. (2009)³⁴ to detect complexes from PPI networks based on repeated merging of maximal cliques. Some earlier algorithms like CFinder³⁵ and Local Clique Merging Algorithm (LCMA)³⁶ also adopted clique merging to find dense neighborhoods, but the distinct advantage of CMC over these algorithms is its ability to work on weighted networks and to find relatively low density regions (in subsequent improved versions of CMC).

CMC begins by enumerating all maximal cliques in the PPI network using the Cliques algorithm proposed by Tomita et al.³⁷. Although enumerating all maximal cliques is NP-hard, this does not pose a problem in PPI networks because these networks are usually sparse. CMC then assigns a score to each clique C based on its weighted density, which considers the reliabilities (weights) of the interactions within the clique:

$$Score(C) = \frac{\sum_{u,v \in C} w(u,v)}{|C|.(|C| - 1)}. \quad (1)$$

CMC then ranks these cliques in decreasing order of their scores and iteratively merges or removes highly overlapping cliques based on their inter-connectivity scores. The inter-connectivity score of two cliques C_i and C_j is based on the non-overlapping regions of the two cliques and is defined as:

$$Inter_score(C_i, C_j) = \sqrt{\frac{\sum_{u \in (C_i - C_j)} \sum_{v \in C_j} w(u,v)}{|C_i - C_j| \cdot |C_j|} \cdot \frac{\sum_{u \in (C_j - C_i)} \sum_{v \in C_i} w(u,v)}{|C_j - C_i| \cdot |C_i|}} \quad (2)$$

CMC determines whether two cliques C_i and C_j sufficiently overlap: $|C_i \cap C_j|/|C_j| \geq overlap_thresh$. If so, C_j is either removed or merged with C_i based on the *inter_score*: if the $inter_score(C_i, C_j) \geq merge_thresh$, then C_i and C_j are merged, else C_j is removed. Finally, all the resultant merged clusters are output as the predicted complexes.

Clustering with Overlapping Neighborhood Expansion (ClusterONE)

Nepusz et al. (2012)³⁸ proposed ClusterONE, a method for detecting overlapping protein complexes from weighted PPI networks, based on seeding and greedy growth, similar to MCODE²⁹. ClusterONE uses a cohesiveness measure to determine how likely a group of proteins form a complex, and is based on the weight of the interactions within the group and with the rest of the network.

To begin with, ClusterONE identifies seed proteins and greedily grows them into groups with high cohesiveness. When the greedy growth for a group cannot progress any more, a next seed protein is selected to repeat the procedure until no more seed proteins remain. In the second step, ClusterONE identifies highly overlapping cohesive groups and merges them into potential complex candidates. ClusterONE allows identification of overlapping complexes if each of the merged groups represent individual complexes that share proteins. Nepusz et al.'s comparisons with methods like MCODE, MCL and CMC showed that the complexes from ClusterONE achieved comparable accuracies when matched against known 'gold standard' complexes and MCL achieved the closest performance to ClusterONE with the exception that MCL produced only non-overlapping clusters - a distinct advantage of ClusterONE.

Some other methods based on graph clustering

Apart from these discussed methods, three other methods worth mentioning here are LCMA (2005)³⁶, PCP (2007)³⁹ and HACO (2009)⁴⁰. The LCMA algorithm first locates cliques within local neighborhoods using vertex degrees and then merges

them based on overlaps to produce complexes. Protein Complex Prediction (PCP) uses FS Weight scoring to remove unreliable interactions and add indirect interactions, and then merges cliques to produce the final list of complexes. On the other hand, HACO uses hierarchical agglomerative clustering to produce the initial set of (non-overlapping) clusters. Proteins are then assigned to multiple clusters based on their interactions to the clusters to produce the final list of overlapping clusters.

A few other recently proposed (2010 - 2011) methods include those by Zhang et al.⁴¹, Ma et al.⁴², Wang et al.⁴³ and Chin et al.⁴⁴. These use the property of “bridgeness” of cross-edges among clusters along with the internal connectivities to detect complexes.

2.3. Methods incorporating core-attachment structure

Gavin and colleagues (2006)⁹ performed large-scale analysis of yeast complexes and found that the proteins with complexes were divided into two distinct groups, “cores” and “attachments”. The cores formed central functional units of complexes, while the attachment proteins aided these cores in performing their functions. Several computational methods were proposed to reconstruct complexes from PPI networks by capitalizing on this structural organization.

Wu Min et al. (2009)⁴⁵ proposed the COACH method which reconstructs complexes in two stages - it identifies dense core regions, and subsequently includes proteins as attachments to these cores. Figure 3 summarizes how COACH identifies core and attachment proteins to build complexes.

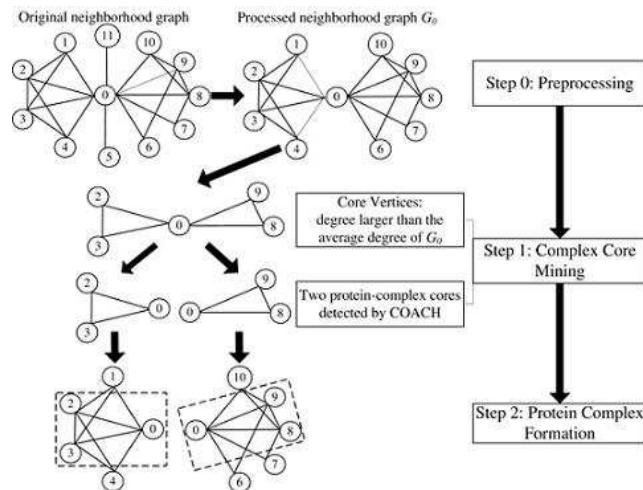


Fig. 3. The identification of core and attachment proteins in COACH: The cores are first identified based on vertex degrees in the neighborhood graphs. Attachment proteins are then appended to these cores to build the final complexes.

Leung et al. (2009)⁴⁶ proposed the CORE method to identify protein cores within the PPI network. They defined the probability of two proteins p_1 and p_2 (of degrees d_1 and d_2 , respectively) to belong to the same core using two main factors: whether the two proteins interact or not and the number of common neighbors m between them. The probability that p_1 and p_2 have $\geq i$ interactions and $\geq m$ common neighbors is calculated under the null hypothesis that d_1 edges connecting p_1 and d_2 edges connecting p_2 are randomly assigned in the PPI network according to a uniform distribution. This probability is used to arrive at a p -value for whether p_1 and p_2 belong to the same core. Subsequently, CORE merges sets of core proteins of sizes two, three, etc. until further increase in size is not possible, to produce the final set of cores. CORE then scores and ranks the predicted cores based on the number of internal and external interactions in them. The attachments are added to these cores in a manner similar to COACH to produce the final set of complexes.

Srihari et al. proposed MCL-CA (2009)⁴⁷ and the improved (weighted) version MCL-CAw (2010)⁴⁸ which identify complexes by refining clusters produced by the MCL algorithm^{31,33} by incorporating core-attachment structure. Essentially, MCL-CAw categorizes proteins within MCL clusters into “core” and “attachment” proteins based on their connectivities, and then selects only these categorized proteins into complexes while discarding the remaining “noisy” proteins. This enables MCL-CAw to “trim” the raw MCL clusters. Further, unlike CORE and COACH, the refinement in MCL-CAw capitalizes on reliability scores assigned to the interactions. Consequently, MCL-CAw reconstructs significantly higher number of ‘gold standard’ complexes ($\sim 30\%$ higher) and with better accuracies compared to plain MCL.

Chin et al. (2010)⁴⁴ proposed the HUNTER algorithm which begins by generating a module seed $MS(v)$ for each node v in the PPI network. $MS(v)$ is then pruned by removing vertices having low weight edges to other members of $MS(v)$. Then the maximal q -connected subnetwork of $MS(v)$ is selected as the initial core $MQC(v)$. This core is then expanded into a module by adding new vertices that share many neighbors with $MQC(v)$. If two modules overlap beyond a certain threshold, these modules are merged. The resultant collection of modules form the final set of predicted complexes.

2.4. Methods incorporating functional information

Proteins within complexes are generally enriched with same or similar functions^{3,9}. If the functional information for proteins from an organism are available, then this information can be combined with topological information from PPI networks for the reconstruction of complexes from the organism. One possible way to incorporate functional information is to score the interactions based on the functional similarity between the interacting pairs of proteins. Alternately, functional annotations (for example, from Gene Ontology⁴⁹) can be used to aid decisions where including or excluding a protein into complexes purely based on topological information might

be difficult.

Restricted Neighborhood Search Clustering (RNSC)

King et al. (2004)⁵⁰ proposed the RNSC algorithm that combines topological and Gene Ontology information to detect complexes. The algorithm operates in two steps - it begins by clustering the PPI network and then filters the clusters based on cluster properties and functional homogeneity.

The network $G = (V, E)$ is first randomly partitioned into multiple subnetworks, which is essentially a partitioning of the node set V . The algorithm then iteratively moves nodes from one cluster to another in a randomized fashion till an integer-valued cost function is optimized. A common problem among such clustering algorithms is the tendency to settle in poor local minima. To avoid this, the RNSC algorithm adopts diversification moves, which shuffle the clustering by occasionally dispersing the contents of a cluster at random. Once the clustering process is completed, clusters of small sizes or densities (the lower bound on cluster sizes and densities are experimentally determined) are discarded. Finally, a p -value is calculated using functional annotations (from GO) for each cluster that measures the functional homogeneity of the clusters. All clusters above a certain p -value are discarded to produce the final list of predicted complexes. Based on experiments, King et al. recommend cluster density cut-off of 0.70 and p -value cut-off of 10^{-3} .

Dense neighborhood Extraction using Connectivity and conFidence Features (DECAFF)

Li et al. (2007)⁵¹ proposed the DECAFF algorithm which essentially is an extention of the LCMA algorithm³⁶ proposed earlier by the same group. DECAFF identifies dense subgraphs in a neighborhood graph using a hub-removal algorithm. Local cliques are identified in these dense subgraphs and merged based on overlaps to produce clusters. Each cluster is assigned a functional reliability score, which is the average of the reliabilities of the edges within the cluster. All clusters with low reliabilities are discarded to produce the final set of predicted complexes.

The PCP algorithm³⁹ described earlier can also be categorized into this set of methods because PCP uses a weighting scheme based on functional similarity (though the similarity is inferred from topology) to assign reliability scores to interactions, and then uses a clique merging strategy to detect complexes.

2.5. *Methods incorporating evolutionary information*

The increasing availability of PPI data from multiple species like yeast, fly, worm and some mammals has made it feasible to use insights from cross-species analysis for detection of (conserved) complexes. The assumption is that proteins belonging to real complexes should generally be conserved through the evolution process to act as an integrated functional unit³.

Sharan et al. proposed methods (2005-2007)^{52,53} for detection of conserved complexes across species based on the evolution of PPI networks. In these methods, an orthology network (network alignment graph) is constructed from the PPI networks of different species, which essentially represents the orthologous proteins and their conserved interactions across the species. For a protein pair $\{u_1, v_1\}$ in network G_1 of species S_1 and $\{u_2, v_2\}$ in G_2 of species S_2 , the orthology network G_{12} contains the pair $\{u, v\}$ if u_1 is orthologous to u_2 , and v_1 is orthologous to v_2 . The edge (u, v) is weighted by the sequence similarities between the pairs $\{u_1, v_1\}$, and $\{u_2, v_2\}$. Any subgraph in this orthology network G_{12} is therefore a conserved subnetwork of G_1 and G_2 . Such candidate subgraphs are then evaluated for parts of conserved complexes. Based on this idea, a tool *QNet*⁵⁴ was developed which returns conserved complexes from different species when queried using known complexes from yeast.

2.6. Methods based on co-operative and exclusive interactions

The overlapping binding interfaces in a protein may prevent multiple interactions involving these interfaces from occurring simultaneously⁵⁵. In other words, the set of interactions in which a protein participates may be either co-operative or mutually exclusive. The information about the co-occurrence or exclusiveness of interactions can therefore be useful for predicting complexes with higher accuracy. This information can be gathered from the interacting domains of protein pairs or the three-dimensional structures of the interacting surfaces.

Ozawa et al. (2010)⁵⁶ proposed a refinement method over MCODE and MCL to filter predicted complexes based on exclusive and co-operative interactions. They used domain-domain interactions to identify conflicting pairs of protein interactions in order to include or exclude proteins within candidate complexes. Based on their results, the accuracies of predicted complexes from MCODE and MCL improved by two-fold.

On the other hand, Jung et al. (2010)⁵⁷ used structural interface data to construct a simultaneous PPI network (SPIN) containing only co-operative interactions and excluding competition from mutually exclusive interactions. MCODE and LCMA algorithms tested on this SPIN displayed a sizeable improvement in correctly predicted complexes.

Even though incorporating information about co-operative and exclusive interactions shows promising improvement in complex detection algorithms, there are still several practical problems related to this approach. Gathering more data on conflicting interactions, especially based on three-dimensional structures of interfaces, needs to be addressed before this approach can be more easily adopted.

2.7. Incorporating other possible kinds of information

In a recent foresighted survey by Przytycka et al.⁵⁸, the application of network dynamics (temporal information) into current computational analysis is discussed

at good lengths, especially with respect to detection of complexes and pathways from protein interaction networks. The authors suggest that if sufficient information about the ‘timing activities’ of proteins can be obtained, the dynamical nature of the underlying organizational principles in interaction networks can be better understood. This shift from static to dynamic network analysis is vital to understanding several cellular processes, some of which may have been wrongly understood due to ignoring dynamic information.

3. Comparative assessment of existing methods

Considering the wide variety of proposed methods for complex detection, one can gauge the seriousness in the research effort towards computational identification and categorization of complexes. Several surveys and experiments^{25,26,27} have focused on the comparative analyses of these proposed methods for complex detection. Each new work on complex detection also comes with detailed comparative analyses of the new method with some earlier methods. However, due to the differences in PPI and benchmark datasets, evaluation criteria, thresholds and parameters used, and the subset of methods considered for these comparative assessments, different works arrive at different results on the relative performance of methods. But, typically the following broadly accepted criteria are used across the works.

If a reasonably large ‘gold standard’ set of complexes is available (as in the case of yeast), the performance of a method can be gauged on how accurately its predicted complexes reconstruct or recover the ‘gold standard’. Two commonly adopted measures for this are precision and recall³⁴. Precision measures how many among the predicted complexes match some ‘gold standard’ complex, in turn measuring the proportion of reliable predictions (accuracy) from the method. Recall measures how many of the ‘gold standard’ complexes are reconstructed by the method, in turn measuring the coverage or sensitivity of the method. Some methods tend to produce too many (sometimes arbitrary) predictions resulting in high recall but very low precision, and therefore too many false positives to consider the method even reasonably reliable. To handle this, a combination of precision and recall, usually through a harmonic mean called F-measure, is used to evaluate how “balanced” is the method.

On the other hand, if a ‘gold standard’ set is not available (as in the case mammals, currently), “self-evaluatory” measures like cluster cohesiveness and separability is used^{29,50}. The cohesiveness of a predicted complex (cluster) usually measures topological characteristics of the cluster, for example, its interaction density or size, while separability measures how separated is the cluster from others²⁹. A combination of cohesiveness and separability reveals how modular is the clustering and therefore how likely the individual clusters represent real complexes.

Another typically independent way to evaluate the predictions is to measure the functional or co-localization coherence of the clusters subjected, however, to availability of appropriate annotation data^{34,50}. This captures how functionally coher-

ent are the proteins within a predicted complex and whether they are co-localized within the cell. The usual annotations required for these calculations are functions and sub-cellular localizations of the proteins. This evaluation is particularly useful for alternative validation of the predictions.

Now, we present a summary of some representative surveys and comparative assessments and their conclusions. One of the first comprehensive assessments was performed by Brohee and van Helden (2006)²⁵. They performed a detailed empirical comparison between MCODE²⁹, MCL³⁰, RNSC⁵⁰ and Super-paramagnetic Clustering (SPC)⁵⁹. These algorithms were tested on PPI datasets from high-throughput experiments, and the resultant complexes were evaluated against benchmark complexes from MIPS⁶⁰. Additionally, the PPI datasets were introduced with artificial noise (random edge addition and deletion) to test the robustness of these algorithms. They concluded that MCL and RNSC outperformed MCODE and SPC in terms of precision (the proportion of correctly predicted complexes) and recall (the proportion of correctly derived benchmarks). RNSC was robust to variation in its input parameter settings, while the performance of the other three varied widely for parameter changes. MCL was remarkably robust even upon introducing 80%-100% random noise. Overall, the experiments confirmed the general superiority of MCL over the other three algorithms.

Vlasblom et al. (2009)²⁶ compared MCL with another clustering algorithm, Affinity Propagation (AP)⁶¹ on unweighted as well as weighted PPI networks. The initial unweighted network was built from a set of 408 hand-curated complexes from Wodak lab⁶³ followed by random addition and removal of edges to mimic real PPI networks. The weighted network was obtained from the Collins et al.'s work¹⁵, generated from Gavin and Krogan datasets^{9,10}. They concluded that MCL performed considerably better than AP in terms of accuracy and separation of predicted clusters, and robustness to random noise. In particular, MCL was able to achieve about 90% accuracy and 80% separation compared to only 70% accuracy and 50% separation of AP on unweighted PPI networks with introduced random noise. MCL was able to discover benchmark complexes even at high (40%) noise levels.

More recently (2010), Li et al.²⁷ performed a detailed comparative evaluation of several algorithms: MCODE²⁹, MCL³⁰, CORE⁴⁶, COACH⁴⁵, RNSC⁵⁰ and DECAFF⁵¹. These algorithms were tested on PPI datasets from DIP⁶² and Krogan et al.¹⁰. The DIP network consisted of 17203 interactions among 4930 proteins, while the Krogan dataset consisted of 14077 interactions among 3581 proteins. They used a total of 428 benchmark complexes from MIPS⁶⁰, Aloy et al.⁶⁴ and SGD⁶⁵. A cluster P from a method was considered a correct match to a benchmark complex B using the Bader score²⁹ $|V_P \cap V_B|^2 / (|V_P| \cdot |V_B|) \geq 0.20$, where V_P denotes the number of proteins in P , and V_B denotes the number of proteins in B . Based on this criteria, the precision, recall and F-measure values were calculated. The comparisons of these algorithms is shown in Figure 4 (adapted from²⁷). The methods are arranged in chronological order, and it is interesting to note that over the years, the F-

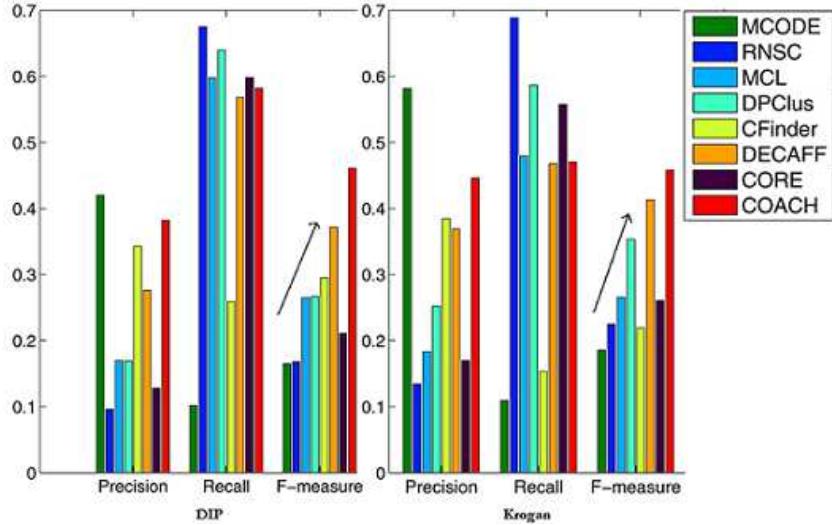


Fig. 4. Comparative performance of complex detection methods in terms of precision, recall and F-measure on DIP and Krogan datasets. The methods are arranged in chronological order, and it is interesting to note that over the years, the F-measures have improved.

measures have improved. Li et al. concluded that MCL, RNSC, CORE, COACH and DECAFF attained the best recall values. MCODE was able to achieve the highest precision, but it produced very few clusters resulting in very low recall.

3.1. Our assessment of some complex detection methods

3.1.1. Preparation of experimental data

In our assessment, we experimentally evaluated some key complex detection methods on both unscored (raw) and scored PPI networks. To build our unscored network, we combined the physical interaction from two TAP-MS experiments, Gavin et al. (2006)⁹ and Krogan et al. (2006)¹⁰, which we call the Gavin+Krogan (G+K) network. We then gathered the scored version of this network, the Consolidated network from Collins et al. (2007)¹⁵. This network comprises of interactions from Gavin et al. and Krogan et al. scored using the Purification Enrichment scheme¹⁵. Some of the properties of these networks are shown in Table 1.

The benchmark (reference or ‘gold standard’) set of complexes was built from three independent sources: 408 complexes of the Wodak lab CYC2008 catalogue⁶³, 313 complexes of MIPS⁶⁰, and 101 complexes curated by Aloy et al.⁶⁴. The properties of these reference sets are shown in Table 2. We considered each of these reference sets independently for the evaluation of the methods. We did not merge them into one comprehensive list of complexes because the individual complex compositions are different across the three sources and some complexes may also get

PPI Network	# Proteins	# Interactions	Avg node degree
Gavin	1430	7592	10.62
Krogan ‘Core’	2708	7123	5.26
Gavin+Krogan	2964	13507	9.12
Consolidated	1622	9704	11.96

Table 1. Properties of the PPI networks used for the evaluation of methods

double-counted (because of different names used for the same complex).

Benchmark	#Complexes	# Proteins	# Complexes of size				Avg density
			< 3	3-10	11-25	> 25	
Wodak	408	1627	172	204	27	5	0.639
MIPS	313	1225	106	138	42	27	0.412
Aloy	101	630	23	58	19	1	0.747

Table 2. Properties of hand-curated (*bona fide*) yeast complexes from Wodak lab, MIPS and Aloy

3.1.2. Metrics for evaluating the predicted complexes

Let $\mathcal{B} = \{B_1, B_2, \dots, B_m\}$ and $\mathcal{C} = \{C_1, C_2, \dots, C_n\}$ be the sets of benchmark and predicted complexes, respectively. We use the Jaccard coefficient J to quantify the overlap between a benchmark complex B_i and a predicted complex C_j :

$$J(B_i, C_j) = \frac{|B_i \cap C_j|}{|B_i \cup C_j|}. \quad (3)$$

We consider B_i to be covered by C_j , if $J(B_i, C_j) \geq \text{overlap threshold } t$. In our experiments, we set the threshold $t = 0.5$, which requires $|B_i \cap C_j| \geq \frac{|B_i| + |C_j|}{3}$. For example, if $|B_i| = |C_j| = 8$, the overlap between B_i and C_j should be at least 6.

We use previously reported³⁴ definitions of *recall* (coverage) and *precision* (sensitivity) of the set of predicted complexes:

$$\text{Recall } Rc = \frac{|\{B_i | B_i \in \mathcal{B} \wedge \exists C_j \in \mathcal{C}; J(B_i, C_j) \geq t\}|}{|\mathcal{B}|} \quad (4)$$

Here, $|\{B_i | B_i \in \mathcal{B} \wedge \exists C_j \in \mathcal{C}; J(B_i, C_j) \geq t\}|$ gives the number of *derived benchmarks*.

$$\text{Precision } Pr = \frac{|\{C_j | C_j \in \mathcal{C} \wedge \exists B_i \in \mathcal{B}; J(B_i, C_j) \geq t\}|}{|\mathcal{C}|} \quad (5)$$

Here, $|\{C_j | C_j \in \mathcal{C} \wedge \exists B_i \in \mathcal{B}; J(B_i, C_j) \geq t\}|$ gives the number of *matched predictions*.

We calculated the *F-measure* as the harmonic mean of precision and recall,

$$F = \frac{2 * Pr * Rc}{Pr + Rc} \quad (6)$$

3.1.3. Experimental evaluation of methods

We considered the following methods for our evaluation:

- On the unscored network, MCL (2002, 2004)^{30,33}, MCL-CA (2009)⁴⁷, MCL-CAw (2010)⁴⁸, CORE (2009)⁴⁶, COACH (2009)⁴⁵, CMC (2009)³⁴ and HACO (2009)⁴⁰;
- On the scored network, MCL (2002, 2004)^{30,33}, MCLO (2007)³², MCL-CAw (2010)^{47,48}, CMC (2009)³⁴ and HACO (2009)⁴⁰.

We do not show comparisons for older methods like MCODE (2003)²⁹ and RNSC (2004)⁵⁰ because these have been evaluated extensively in several earlier surveys^{25,27}, instead we included MCL as a benchmark in all our comparisons since MCL has been repeatedly shown to perform better than these older methods^{25,26,27}. Further, not all methods are devised to make use of interaction confidence scores, and therefore we selected only the ones capable of doing so for the evaluations on the scored network.

Table 3 shows the precision and recall values for methods evaluated on the unscored Gavin+Krogan network across the three benchmark sets. The table shows that CORE, HACO and MCL-CAw performed significantly better in terms of recall compared to the rest of the methods. In particular, MCL-CAw performed considerably better than plain MCL indicating that incorporating core-attachment structure into raw MCL clusters helped to improve the accuracies of the predicted complexes. This indicated that incorporating some kind of biological knowledge helped to identify complexes more accurately.

Next, Table 4 shows these values for the methods evaluated on the scored Consolidated network. This table shows that all methods were able to reconstruct significantly higher number of complexes upon scoring as compared to on the unscored network. This clearly indicated that noise in raw datasets (negatively) impacted the performance of methods, and reliability scoring aided in alleviating this impact and thereby improving the performance of methods. This demonstrated the effectiveness of current reliability scoring schemes in cleaning raw interaction datasets for focused studies such as complex detection.

3.1.4. Plugging experimental results into our taxonomy

We next “plugged-in” these evaluation results as well as results obtained from some earlier surveys^{26,27} into our “bin-and-stack” classification, as shown in Figure 5. For

The unscored Gavin+Krogan network
 #Proteins 2964; #Interactions 13507

		#Predicted	Method						
			MCL	MCL-CA	MCL-CAw	COACH	CORE	CMC	HACO
Wodak (#182)	#Matched	242	219	130	447	386	113	278	
	Precision	55	49	69	62	83	60	78	
	#Derived	0.226	0.224	0.531	0.139	0.215	0.531	0.281	
	Recall	62	49	75	49	83	60	85	
MIPS (#177)	#Matched	0.338	0.269	0.412	0.269	0.456	0.330	0.467	
	Precision	35	42	42	45	59	41	45	
	#Derived	0.143	0.192	0.323	0.101	0.153	0.363	0.162	
	Recall	40	42	53	38	59	41	57	
Aloy (#76)	#Matched	0.226	0.237	0.300	0.215	0.333	0.232	0.322	
	Precision	43	41	47	54	59	43	59	
	#Derived	0.179	0.187	0.362	0.121	0.153	0.381	0.212	
	Recall	42	41	52	37	59	43	59	

Table 3. Comparisons between different methods on the unscored Gavin+Krogan network. CORE showed the best recall followed by HACO and MCL-CAw.

The Consolidated_{3.19} network
 #Proteins 1622; #Interactions 9704

		#Predicted	Method				
			MCL	MCLO	MCL-CAw	CMC	HACO
Wodak (#145)	#Matched	116	119	130	77	101	
	Precision	70	80	83	67	57	
	#Derived	0.603	0.672	0.638	0.870	0.564	
	Recall	79	80	90	67	64	
MIPS (#157)	#Matched	0.545	0.552	0.621	0.462	0.441	
	Precision	48	65	53	56	40	
	#Derived	0.414	0.546	0.408	0.727	0.396	
	Recall	63	65	67	56	57	
Aloy (#76)	#Matched	0.401	0.414	0.427	0.357	0.363	
	Precision	54	56	57	45	44	
	#Derived	0.466	0.471	0.438	0.584	0.436	
	Recall	55	56	55	45	45	

Table 4. Comparisons between the different methods on the Consolidated_{3.19} network. MCL-CAw showed the best recall followed by CMC.

each method, we show the F-values *before / after* scoring, that is, on the unscored G+K network and the scored Consolidated network. Such a representation in our classification revealed two interesting insights,

- (1) incorporating biological information in addition to PPI topology improved performance of the methods (the F measures have increased in the vertical layers

compared to the lowest layer);

- (2) reliability scoring significantly improved performance of the methods, as shown by the before-after values.

This representation also shows how complex detection methods have evolved over the years to improve performance, and therefore our taxonomy can be useful to guide future directions for further improvement.

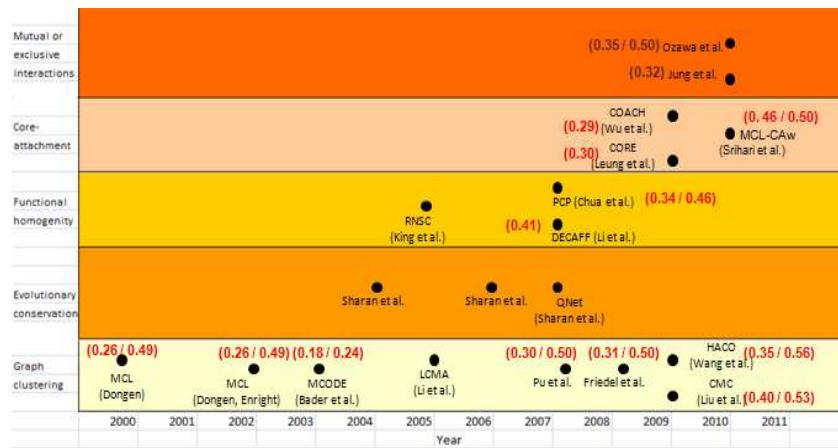


Fig. 5. Plugging-in F-values (before-after scoring) of existing methods into our Bin-and-Stack classification. Incorporating biological information and affinity scoring significantly boosts performance.

4. Open challenges in complex detection from PPI networks

The review and evaluation of computational methods in the above sections reveal several critical challenges facing accurate identification of complexes from high-throughput interaction datasets. We saw that most methods are considerably impacted by noise in raw datasets. Further, most methods are able to reconstruct only a fraction of the known complexes (achieve at the most 65% recall) even upon scoring. This points towards some inherent limitations within the methods itself. On this basis, we broadly classify the challenges facing current methods into two categories, (i) challenges originating from biological datasets; (ii) challenges originating from existing computational techniques.

4.1. Challenges from interaction datasets

Even though over the last few years, several independent high-throughput experiments have helped to catalogue enormous amount of interactions from yeast, they

show surprising lack of correlation with each other, and lack of coverage - bias towards high abundance proteins and against proteins from certain cellular compartments (like cell wall and plasma membrane)^{12,11,13,14}. Also, each dataset still contains a substantial number of false positives (noise) that can compromise the utility of these datasets for more focused studies like complex detection, as seen from our evaluation results. In order to reduce the impact of such discrepancies, a number of data integration and reliability scoring schemes have been devised^{15,16,17,18,19,20,21,22,23,24}.

To overcome these challenges to some extent, in our evaluation, we combined multiple datasets (from Gavin et al.⁹ and Krogan et al.¹⁰) to account for the lack of interaction coverage, and also adopted scoring prior to predicting complexes. In spite of these precautionary steps, we notice that most methods are able to reconstruct only a fraction of the known complexes, and we still have a long way to go towards identification of meaningful novel complexes through computational means.

4.2. Challenges from existing complex detection methods

As noted earlier, even though there have been numerous methods developed for complex detection, most of them suffer from low recall (at most 65% recall even on the scored network; Table 4). Even a “union” of these methods achieves at most 70-75% recall on average across a variety of PPI datasets⁶⁶. One of the crucial reasons for this limitation is that every method, in one way or another, relies on the key assumption that complexes are embedded among “dense” regions of the network. However, recent experiments have indicated that relying too much on this assumption in the wake of insufficient credible interaction data causes these methods to miss many complexes that are of low densities, that is, “sparse” in the network⁶⁶. Therefore the need is to find alternative ways to model complexes than mere dense subnetworks, and also to compensate for the sparsity of topological information by augmenting other kinds of biological information.

4.2.1. Detection of sparse complexes

In the attempt to detect sparse complexes, the recent work by Srihari et al. (2012)⁶⁶ is insightful and can guide further directions towards tackling this challenge. Srihari et al. (2012)⁶⁶ noticed that most existing computational methods based on PPI networks rely overly on the assumption that complexes are embedded among “dense” regions of the network, and therefore miss most of the “sparse” complexes that have very low interaction densities or lie disconnected in the network. These complexes are missed by current methods due to the lack of crucial topological information (missing interactions and/or proteins) - for example, even in the well-studied organism yeast, only $\sim 70\%$ of the interactome has been validated and catalogued¹³. In order to overcome these “topological gaps”, the authors proposed careful augmenting of functional interactions to PPI networks. Functional interactions represent

logical or conceptual associations among proteins, and therefore “encode” a variety of biological similarities or affinities among proteins beyond just physical interactivity, thereby compensating for the lack of topological information in PPI networks. In order to do this augmentation systematically they proposed the SPARC algorithm.

SPARC selects low quality clusters predicted from the physical (PPI) network using existing methods, and then selectively enhances these clusters to reconstruct (sparse) complexes. The key idea is that many of these low quality clusters are in fact fractions (or “pieces”) of sparse complexes embedded in the PPI network, but due to missing interactions they lie “scattered” in the network and do not represent whole complexes in their current forms. If these clusters can be carefully enhanced by augmenting functional interactions, then several of the sparse complexes can be reconstructed accurately. This enhancement involves increasing their interaction densities and “pulling-in” together their disconnected components. However, during the selection of the initial set of low quality clusters, many may just represent noisy predictions (false positives). In order to determine the clusters that are more likely to represent complexes, SPARC makes use of a novel Component-Edge (CE) score. The CE-score is a topological measure combining connectivity and relative density of the clusters, and is shown to more accurately correlate with the topological characteristics of real complexes compared to traditionally accepted measures like edge density. The CE-score is calculated for every low quality cluster predicted from the PPI network. SPARC then augments functional interactions to the clusters and checks if their CE-scores improve beyond a certain threshold. If a cluster shows the required improvement, it is output as a potential candidate representing a sparse complex. Srihari et al. showed through extensive experiments on clusters produced from methods like MCL^{30,33}, MCL-CAw⁴⁸, CMC³⁴ and HACO⁴⁰ that SPARC was capable of improving the overall recall of these methods by upto 47% on average across a variety of networks. Specifically, SPARC helped to reconstruct 25% more complexes among the ones that were sparse.

4.2.2. *Detection of small and temporal complexes*

Small complexes (complexes of two or three proteins) also pose severe challenges in identification, particularly if PPI network topology is the only available information. In fact most complex detection methods based solely on the PPI network only attempt to identify complexes with at least 4 or more proteins in the network^{34,46,48}. The attempt to predict small complexes (pairs or triplets) from the network based only on connectivity typically produces a significant fraction of false positives. Further, the smaller the size of a complex, the more prone it is to sparsity - missing even a few interactions can result in very low densities or render the complexes disconnected. Due to these challenges, additional information apart from PPI network topology is required for detection of such complexes.

In a recent work (2012), Srihari et al.⁶⁷ incorporated temporal information to identify small complexes. The authors focused on identifying small complexes

that are assembled during the yeast cell cycle in a temporal manner. They noticed that several high-degree proteins such as kinases interacted with different subsets of proteins during different phases of the yeast cell cycle to assemble multiple phase-specific complexes. However, due to the lack of temporal information to disambiguate (segregate) the different phase-specific complexes, existing topology-based methods produced large clusters of complexes fused together. Srihari et al. decomposed such large clusters by incorporating temporal information on the yeast cell cycle in which each protein showed peak expression, and thereby segregated the individual constituent complexes. Many of the segregated complexes were small and represented complexes of kinases and their temporal substrates.

Srihari et al. also noticed that by incorporating such temporal information the “dynamics” of protein complexes could be better understood. They observed an interesting relationship between the “staticness” (constitutive expression) of a protein and its participation in multiple complexes⁶⁷ - cells tend to maintain generic proteins as ‘static’ to enable their “reusability” across multiple temporal complexes.

4.3. Challenges in detecting membrane complexes

Membrane protein complexes are formed by physical interactions among membrane proteins. Membrane proteins are attached to or associated with the membranes of the cell or its organelles. Membrane proteins constitute approximately 30% of the proteomes of organisms, yet they are one of the least studied subsets of proteins. The study of membrane proteins and their complexes is crucial in understanding diseases and aiding new drug discoveries⁶⁸.

Membrane protein complexes are notoriously difficult to study using traditional high-throughput techniques like Y2H and TAP-MS⁶⁹. This is due in part to the hydrophobic (insoluble) nature of membrane proteins, as well as the ready dissociation of subunit interactions, either between transmembrane subunits or between transmembrane and cytoplasmic subunits^{69,70}.

In order to counter the disadvantages of conventional techniques, new biochemical techniques have been developed recently to facilitate the characterization of interactions among membrane proteins. Among these is the split-ubiquitin membrane yeast two-hybrid (MYTH) system^{71,72,73}. With the development of the high-throughput MYTH system, a fair number of interactions among membrane proteins have been recently catalogued in species such as *Arabidopsis thaliana*⁶⁹ and yeast *Saccharomyces cerevisiae*^{71,72}.

The identification of membrane complexes requires understanding their assembly - how the individual proteins come together to form complexes, and how these complexes are eventually degraded. This is because membrane proteins are not stable entities as their soluble counterparts. Studies reveal that this assembly occurs in an orderly fashion, that is, membrane complexes are formed by an ordered assembly of intermediaries, and in order to prevent unwanted intermediaries, this assembly is highly aided by chaperones⁷⁴. Many membrane complexes are formed by transient

interactions involving exchange of proteins in and out of existing complexes via membranes - a phenomenon called 'dynamic exchange'⁷⁴. The need therefore now is to develop sophisticated algorithms that take into account these aspects specific to membrane complexes to mine them effectively from membrane sub-interactomes.

5. Conclusions

Protein complexes are the fundamental functional units responsible for many biological mechanisms within the cell. Their identification is therefore necessary to understand the cellular organization and machinery. The advent of high-throughput techniques for inferring protein interactions in a large-scale fashion has fueled development of computational techniques to systematically mine for potential complexes from the network of interactions. In this work, we reviewed, classified and evaluated some of the key computational methods developed till date for the detection of protein complexes from PPI networks. We presented two insightful taxonomies of existing methods - 'bin-and-stack' and 'tree'. From these taxonomies we note that scoring of raw interaction datasets (followed by filtering of false positives) and integrating key biological insights with topology can significantly improve complex prediction.

Even though more than 20 methods have been developed over the years, complex detection still requires careful attention in handling errors and noise in experimental datasets, and reconstructing complexes with high accuracies. On this front, we identified some of the crucial limitations and challenges facing current experimental and computational techniques. Interaction datasets coming from different experimental sources show surprising lack of correlation and also contain sizeable fraction of spurious (false positive) interactions. This severely impacts the accuracy and coverage of complex detection methods. Further, computational methods also overly rely on the assumption that complexes are embedded among densely connected groups of proteins, an assumption that is not fully valid in the wake of insufficient credible interactions. Finally, the interactions among membrane proteins have not been catalogued adequately making it difficult to identify an important group of complexes necessary for understanding diseases - membrane complexes.

We hope that our review and assessment of computational methods as well as the challenges highlighted here will provide valuable insights to drive future research for further advancing the 'state-of-the-art' in computational prediction, characterization and analysis of protein complexes from organisms.

Acknowledgments

This work was supported in part by the National University of Singapore under ARF grants R-252-000-361-112 and R-252-000-461-112. SS is now associated with the Institute of Molecular Bioscience, University of Queensland, and would like to thank the institute for providing the resources to complete this work.

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